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ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES OF THE AQUEOUS LEAF EXTRACT OF *ARTEMESIA VULGARIS* IN EXPERIMENTAL ANIMAL MODELS

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ABSTRACT

Artemisia vulgaris is a medicinal plant used for the treatment of various risk factors like analgesia, inflammation, ulcers, and wounds. The aqueous extract of the leaf was investigated for its anti-inflammatory and analgesic activities in animal models. The extract at 100, 200 and 400mg/kg body weight reduced significantly the formation of oedema induced by formalin and histamine. In the acetic acid-induced writhing model, the extract had a good analgesic effect characterized by a reduction in the number of writhes when compared to the control. Similarly, the extract caused dose-dependent decrease of licking time and licking frequency in rats injected with 2.5% formalin. These results were also comparable to those of ibuprofen, the reference drug used in this study. Acute toxicity test showed that the plant may be safe for pharmacological uses. This study has provided some justification for the folkloric use of the plant in several communities for conditions such as stomachache, pain and inflammations.

Key words: analgesic, anti-inflammatory, oedema, formalin, histamine, ibuprofen, extract, rats.

INTRODUCTION

Inflammation and pain is a local response of living mammalian tissues to the injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agents. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Oedema formation, leukocyte infiltration and granuloma formation represent such components of inflammation (Mitchell and Cotran, 2000). Oedema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and/or the mediators that increase blood flow (Ialenti *et al.*, 1995).

Drugs which are in use presently for the management of pain and inflammatory conditions are either

narcotics e.g. opioids or non-narcotics e.g. salicylates and corticosteroids e.g. hydrocortisone. All of these drugs possess well known side and toxic effects. Moreover, synthetic drugs are very expensive to develop and whose cost of development ranges from 0.5 to 5 million dollars. On the contrary many medicines of plant origin had been used since long time without any adverse effects.

Therefore, a systematic approach should be made to find out the efficacy of plants against inflammation so as to exploit them as herbal anti-inflammatory agents. The enzyme, phospholipase A₂, is known to be responsible for the formation of mediators of inflammation such as prostaglandins and leukotrienes which by attracting polymorpho nuclear leucocytes to the site of inflammation would lead to tissue damage probably by the release of free radicals. Phospholipase

A2 converts phospholipids in the cell membrane into arachidonic acid, which is highly reactive and is rapidly metabolized by cyclooxygenase (prostaglandin synthesis) to prostaglandins, which are major components that induce pain and inflammation (Higgs *et al.*, 1984; Vane, 1971).

The present study was used to evaluate acute toxicity, anti-inflammatory, analgesic activity of *Artemesia Vulgaris* in swiss wistar rats.

MATERIALS AND METHOD

Collection of Plant Material:

The leaves were collected from the talakona forest, Andhra pradesh, India during month of January 2016 and its identity as *Artemesia Vulgaris* was confirmed by Assistant professor in the department of botany, Sri venkateswara University, Tirupati.

Preparation of extracts and Phytochemical screening

Extraction was done by using soxhlet apparatus with 70% ethanol(hydro alcoholic) as solvent. The extracts were concentrated under reduced pressure dried and stored at 4c temp in air tight containers for further studies.

Phytochemical screening:

The ethanolic extract of A. Vulgaris was analyzed for preliminary phytochemical screening by using spray reagents: Liebermann–Burchard spray reagent (Briekskorn and Capuano, 1953) was used to detect the presence of steroids and pentacyclic triterpenes in the form of violet spots. Neutral ferric chloride was used to detect phenolic compounds that appear in the form of blue spots. Shinoda test (Shindo, 1928) and Fiegl' test (Feigl, 1954) was used to detect flavonoids and glycoside, respectively. The extract was found to be devoid of alkaloids as detected by the DragonDroff's reagent (Bollinger *et al.*, 1965). Further isolation and characterization of pure compounds from the extract is in progress.

PHARMACOLOGICAL SCREENING

Animals used

Wistar rats of either sex, weighing 150 - 200 gm, were procured from the animal house of Venkateswara Enterprises, Bangalore, Karnataka, India. Animals were kept in polypropylene cages and fed on standard laboratory diet (Lipton India Ltd) with water *ad libitum*, maintained at an ambient temperature of 26 ±2⁰c. The ethical clearance was obtained by the Institutional Animal Ethics committee.

Chemicals:

Diclofenac sodium was obtained from Sigma-Aldrich, Bangalore. Formalin, Acetic acid were obtained from SD fine chemicals Ltd Mumbai. and all other reagents used were of analytical grade.

Instruments:

Plethysmometer, and electronic balance (Shimadzu, Model no: DS-852 J).

Acute toxicity study

Wistar rats of either sex, weighing 150 - 200 g and of 90 days age were used to evaluate acute toxicity of the aqueous extract. Animals were then administered by oral route with aqueous extract (50 - 4000 mg / kg), suspended in 2% w/ v gum acacia solution (vehicle). Control group received only vehicle. The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD), revised draft guidelines 423, received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. The aqueous extract was found to be nontoxic up to dose of 4000 mg/kg body weight and hence 1/10 of this dose was taken as the screening dose.

Treatment schedule

As extract (100, 200, 400 mg/kg body weight) and standard anti-inflammatory and analgesic drug diclofenac sodium (10 mg/kg body weight) (sigma-aldrich chemicals, bangalore) was prepared in 1% sodium carboxymethylcellulose (cmc) vehicle as suspension and administered orally. Animals were randomized into three groups, each carrying 6 rats.

1. Experimental group, treated with different doses of extract.
2. Experimental group treated with diclofenac sodium.
3. Control group of animals that were treated with vehicle similar to experimental group.

Anti-inflammatory study:

In this experiment, formalin-induced rat hind paw oedema was used as the animal model of acute inflammation according to Winter *et al.*, 1962 and described previously (Saha *et al.* 2007). Briefly, acute inflammation was produced by sub-plantar injection of 0.1ml of 1% suspension of Formalin with 2% gum acacia in normal saline, in the right hind paw of the rats 1h after the oral administration of test materials. The paw volume was measured by plethysmometer (Ugo Basile, Italy) at 1, 2, 3, and 4 h after the Formalin injection. The extract was administered at 100,200 and 400 mg/kg body weight. Diclofenac 10 mg/kg body weight was used as standard anti-inflammatory agent.

Table 1: Phytochemical screening.

S.no	Phyto chemical	Ethanollic extract of <i>Artemesia Vulgaris</i>
1	Alkaloids	- ve
2	Catachols	-ve
3	Flavanoids	+ ve
4	Phenolic compounds	+ ve
5	Saponins	- ve
6	Steroids	+ ve
7	Tannins	- ve
8	Triterpinoids	+ ve
9	Glycosides	+ ve

Table 2: Effect of *Artemesia Vulgaris* and diclofenac sodium on percent inhibition of Formalin induced paw edema.

S.N O	Groups	Increase in paw diameter(cm) Mean±SEM				
		0hr	1hr	2hr	3hr	4hr
1	Control	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00
2	Diclofenac sodium(10mg/kg)	2.15±0.02	4.05±0.064	4.45±0.028	4.225±0.047	3.70±0.040
3	<i>A. Vulgaris</i> (100mg/kg)	2.2±0.040	2.675±0.047** *	2.4±0.040***	2.25±0.028***	2.12±0.025** *
4	<i>A. Vulgaris</i> (200mg/kg)	2.275±0.0	3.2±0.070***	3.225±0.075** *	3.17±0.062***	3.0±0.040***
5	<i>A. Vulgaris</i> (400mg/kg)	2.25±0.02	2.9±0.040***	2.675±0.085** *	2.375±0.047** *	2.2±0.040***

All values are shown as mean ±SEM and n=6. *** p<0.0001 when compared with control (II) group

Table 2: Effect of *Artemesia Vulgaris* and diclofenac sodium on percent inhibition of Formalin induced paw edema

S.NO	Groups	Writhings mean±S.E	%Inhibition
1.	Control	41.50±0.6455	
2.	Diclofenac sodium(10mg/kg)	12.50±0.6455***	72.89%
3.	<i>A. Vulgaris</i> (100mg/kg)	26.25±0.6292***	34.33%
4.	<i>A. Vulgaris</i> (200mg/kg)	22.25±0.4787***	45.78%
5.	<i>A. Vulgaris</i> (400mg/kg)	13.50±0.6455***	69.87%

All values are shown as mean ±SEM and n=6. *** p<0.0001 when compared with control (II) group

Acetic acid induced writhing test:

The peripheral analgesic activity of leaf extract of *Artemisia Vulgaris* was measured by the acetic acid induced writhing test as described earlier (Saha *et al.*, 2007). Briefly, the inhibition of writhing produced by the plant extract was determined by comparing with the inhibition produced by the control group. Diclofenac at oral dose of 10 mg/kg was used as standard analgesic agent. Intraperitoneal injection of acetic acid (0.7%) at a dose of 0.1 ml/10g of body weight was used to create pain sensation. The number of writhings was calculated for 10 min, 10 min after the application of acetic acid.

STATISTICAL ANALYSIS:

Data were analyzed by one-way ANOVA followed by Dunnet's test and *P* values <0.0001 were considered statistically significant.

RESULTS & DISCUSSION

Acute toxicity:

Acute toxicity studies show that drug is safe up to the dose of 4000 mg/kg body weight. No mortality was observed at 14th day of the acute toxicity study.

Phytochemical Screening:

All extracts obtaining during successive extraction of *Artemisia Vulgaris* Leaves was examined for the presence of various phytoconstituents by performing qualitative phytochemical tests and the results are recorded in table 1.

Anti-inflammatory study: The results were tabulated in table 2

Acetic acid induced writhing test: The results were tabulated in table 3

CONCLUSION

This study has provided some justification for the folkloric use of the plant in several communities for conditions such as stomachache, pain and inflammations.

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